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Use of mobile phase 18-crown-6 to improve peak resolution between mono- and divalent metal and amine cations in ion chromatography

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Abstract

It is difficult to quantify NH_4^+ by ion chromatography in the presence of high concentrations of Na^+ due to peak overlap. The Dionex IonPac CS15 column, which contains phosphonate, carboxylate, and 18-crown-6 functional groups, was originally developed to overcome this problem. We have found that the addition of 18-crown-6 to the eluent promotes improved peak resolution between Na^+ and NH_4^+ even at concentrations as high as 60,000 to 1 using this column. Its use also improves the separation of alkali and alkaline earth metal and amine cations. Mobile phase 18-crown-6 increased the retention times of $CH_3NH_3^+$, NH_4^+, and K^+, and decreased the retention time of Sr^{2+}. The retention times of Li^+, Na^+, Mg^{2+}, Ca^{2+}, $(CH_3)_2NH_2^+$, and $(CH_3)_3NH^+$ were not affected. This method makes possible the direct analysis of ammonia from nitrogenase, the enzyme responsible for biological nitrogen fixation. The resolution of the NH_4^+ peak from the Na^+ and Mg^{2+} peaks improved from zero resolution to values of 6.19 and 5.65, respectively. This technique considerably reduces the analysis time of NH_4^+ in the presence of high concentrations of Mg^{2+} and Na^+ over traditional indophenol measurements.

Keywords: Peak resolution; Mobile phase composition; Ion chromatography; 18-Crown-6; Sodium; Ammonium; Nitrogenase

1. Introduction

In recent years, macrocyclic ligands have been used in ion chromatography as components of both the stationary and mobile phases [1–17]. The interest in these molecules derives from their selectivity in binding specific cations. Our lab has focused on the use of macrocycles in ion chromatography in the separation of both cations and anions [18–26].

Nakagawa et al. [10–13] investigated the use of crown ethers in the mobile phase for the separation of amine cations, peptides, β-lactam antibiotics, and sulfonic acids. They showed that the increase in capacity factors is related to the stability of the complex for those compounds that form complexes with the crown ether. They also showed that the concentration of crown ether influences the capacity factors for some compounds.

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Table 1
Log K values for binding of mono- and divalent, and amine cations with 18-crown-6 in water at 25 °C

Cation	Log K[a]
Li^+	–
Na^+	0.80
K^+	2.23
NH_4^+	1.22
Mg^{2+}	–
Ca^{2+}	0.53
Sr^{2+}	2.75
$CH_3NH_3^+$	1.13
$(CH_3)_2NH_2^+$	–
$(CH_3)_3NH_3^+$	–

(–) Values not reported (sometimes too small to measure).
[a] From [27].

Ohta et al. describe how the addition of macrocycles, particularly 18-crown-6 (1,4,7,10,13,16-hexaoxacyclooctadecane) to the mobile phase improves peak resolution between mono- and divalent cations using an unmodified silica gel column [1–5]. The improved resolution is due to adsorption of 18-crown-6 onto the stationary phase and the formation of macrocycle-cation complexes with selected metal ions [1–6]. The log K values in Table 1 indicate those metal ions that bind 18-crown-6 preferentially [27].

Recently, Dionex developed the IonPac CS15 analytical column, which like its precursor the CS12A, contains phosphonate and carboxylate functional groups, but in addition contains 18-crown-6 groups [15–17]. One of the specific purposes for the development of the IonPac CS15 analytical column was to separate ammonium ion at low concentrations in the presence of high sodium ion concentrations, since such concentration ratios are found commonly in environmental samples [15,16]. Conversely, in power industry samples, it is often necessary to determine low levels of Na^+ in the presence of high levels of NH_4^+. In this column, 18-crown-6 functional groups have been covalently bound to the stationary phase and facilitate the determination of sodium to ammonium concentration ratios greater than 4000 to 1.

The purpose of this study was to demonstrate and quantify the beneficial effects of adding 18-crown-6 to the mobile phase when using the IonPac CS15 analytical column. The retention time and resolution between alkali, alkaline earth, and amine cations was evaluated. These are commonly separated by ion chromatography, and the described method adds versatility towards separating as many ions as possible. Sr^{2+} was evaluated due to its anomalous behavior. It was found that mobile phase 18-crown-6 modifies the retention times of those cations that have been shown to complex with this ligand in water, while others remain unchanged. Since the ammonium ion was retained longer and the sodium ion peak remained virtually unchanged, the separation of Na^+ and NH_4^+ at concentration ratios as high as 60,000 to 1 was made possible.

Optimizing the separation of Na^+ and Mg^{2+} from NH_4^+ has specific application in the determination of NH_3 from nitrogenase by ion chromatography. Analysis of NH_4^+ by ion chromatography significantly reduces the traditional analysis time, as well as the amount of sample required to make the analysis.

2. Experimental

2.1. Materials

IonPac CG12A, CS12A, CG15 and CS15 4 mm analytical columns from Dionex Corp. (Sunnyvale, CA, USA) were used throughout this work. All of the mono- and divalent metals for this work were reagent grade nitrate metal salts (except for calcium and magnesium, which were chlorides) and were purchased from Fisher (Fair Lawn, NJ, USA) or Aldrich (Milwaukee, WI, USA). The amines were purchased from Aldrich in 40 wt.% solution in water. The 18-crown-6 was purchased from Aldrich. Concentrated sulfuric acid was reagent grade and was purchased from EM Industries Inc. (Gibbstown, NJ, USA).

Water used in making the standards and eluents was purified to 18 MΩ resistivity with a Milli-Q water purification system (Millipore). Sparging with helium degassed all eluents.

2.2. Instrumentation

A Dionex DX500 ion chromatograph with an ED40 electrochemical detector and a GP40 gradient pump were used throughout this work. A CSRS-Ultra 4 mm suppressor was used in all separations. The recycle mode was used when the eluent was free from organic additives. The external water mode was employed when 18-crown-6 was added to the mobile

phase. All data were collected and analyzed using a Dionex PeakNet chromatography workstation.

3. Results and discussion

The effect of addition of 18-crown-6 to the eluent using the CS15 column was compared to separations made under similar conditions when using the CS12A column, a comparable column which contains no bound 18-crown-6 functional groups.

3.1. Effect of mobile phase 18-crown-6 on the IonPac CS12A column

The IonPac CS12A analytical column contains phosphonate and carboxylate functional groups, but no bound 18-crown-6. Fig. 1a shows an example of how the retention times of K^+ and Sr^{2+} increased with time when using 0.1 mM 18-crown-6 in the mobile phase with 10.80 mM H_2SO_4. Even after an equilibration time of 4 h of continuous operation, K^+ and Sr^{2+} continued to migrate to longer retention times with each injection. The NH_4^+ and $CH_3NH_3^+$ peaks (not shown for clarity) migrated in a similar fashion, while the retention times of the other cations of interest remained unchanged. At 4 mM 18-crown-6 (Fig. 1b) the retention time of Sr^{2+} was 86 min and K^+ was 60 min. This result confirms Ohta's conclusion that mobile phase 18-crown-6 absorbs to the stationary phase of the column, creating additional retention sites for cation binding [1–5].

The addition of 18-crown-6 to the mobile phase using the IonPac CS12A column is not practical because it requires several hours for the column to reach equilibrium. Thus, we observed that it was difficult to obtain reproducibility at low concentrations of mobile phase 18-crown-6. Furthermore, higher concentrations of mobile phase 18-crown-6 caused the K^+ and Sr^{2+} peaks to broaden and retention times to significantly increase.

3.2. Addition of 18-crown-6 to mobile phase with the IonPac CS15 column

A typical separation of the alkali, alkaline earth, and amine cations with the IonPac CS15 analytical column using 10.80 mM H_2SO_4 as the eluent is shown

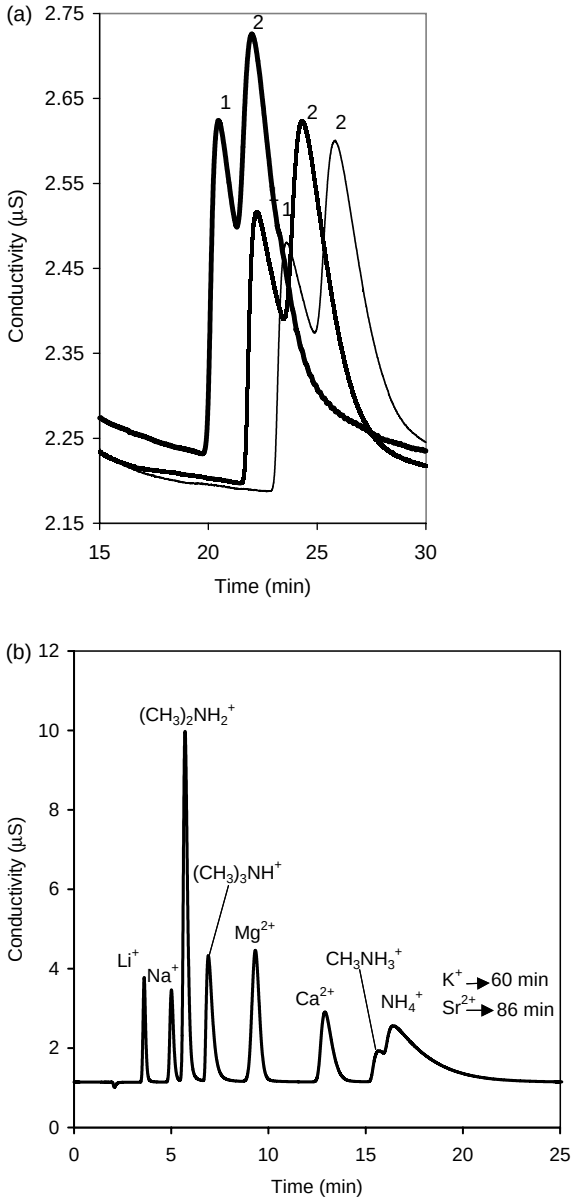

Fig. 1. (a) Migrating K^+ and Sr^{2+} retention times with the addition of 0.1 mM 18-crown-6. Column: CG12A and CS12A. Eluent: 10.80 mM H_2SO_4 and 0.1 mM 18-crown-6. Flow rate: 1 ml min^{-1}. Detection: suppressed conductivity external water mode. Peaks: (1) K^+ (5.00 mg l^{-1}); (2) Sr^{2+} (8.50 mg l^{-1}). Time of day: (▬) 12:17 p.m.; (▬) 1:32 p.m.; (—) 3:07 p.m. (b) Addition of 4 mM mobile phase 18-crown-6. Peaks: Li^+ (0.54 mg l^{-1}); Na^+ (2.00 mg l^{-1}); $(CH_3)_2NH_2^+$ (19.30 mg l^{-1}); $(CH_3)_3NH^+$ (17.70 mg l^{-1}); Mg^{2+} (5.10 mg l^{-1}); Ca^{2+} (5.00 mg l^{-1}); $CH_3NH_3^+$ (16.50 mg l^{-1}); NH_4^+ (2.75 mg l^{-1}).

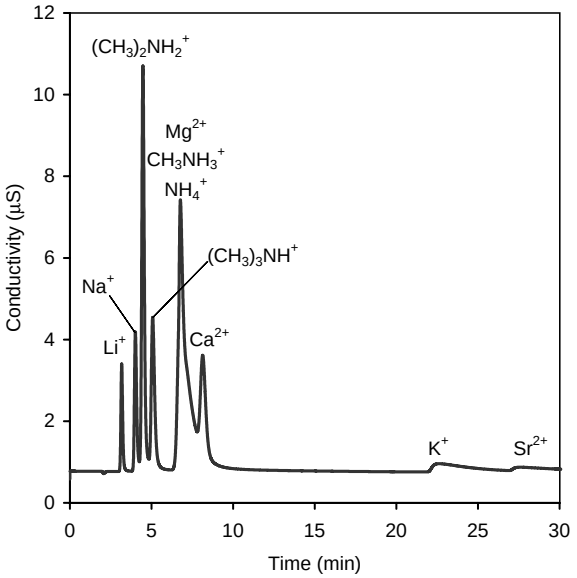

Fig. 2. Traditional cation separation. Column: IonPac CG15 and CS15. Eluent: 10.80 mM H_2SO_4. Flow rate: 1 ml min^{-1}. Detection: suppressed conductivity recycle mode. Peaks: Li^+ (0.54 mg l^{-1}); Na^+ (2.00 mg l^{-1}); $(CH_3)_2NH_2^+$ (19.30 mg l^{-1}); $(CH_3)_3NH^+$ (17.70 mg l^{-1}); Mg^{2+} (5.10 mg l^{-1}), $CH_3NH_3^+$ (16.50 mg l^{-1}); NH_4^+ (2.75 mg l^{-1}); Ca^{2+} (5.00 mg l^{-1}); K^+ (5.00 mg l^{-1}); Sr^{2+} (8.50 mg l^{-1}).

in Fig. 2. The cations eluted in the order $Li^+ < Na^+ < (CH_3)_2NH_2^+ < (CH_3)_3NH^+ < Mg^{2+} < CH_3NH_3^+ = NH_4^+ < Ca^{2+} < K^+ < Sr^{2+}$. Experiments were designed to determine the effects of the addition of 18-crown-6 to the eluent for this system. The acid concentration was maintained at 10.80 mM H_2SO_4, while the concentration of 18-crown-6 in the eluent was varied from 0 to 15 mM. Unlike the CS12A, the CS15 column reached equilibrium quickly to give reproducible chromatograms even at lower concentrations of mobile phase 18-crown-6. Thus, the problem with varying retention times upon repeated injections that was observed with the CS12A column did not appear with the CS15 column. The bound 18-crown-6 groups on the CS15 column are clearly making the difference. Fig. 3a and b shows the retention times in the isocratic separation of the above mentioned cations at varying 18-crown-6 concentrations. As predicted by the thermodynamic data (Table 1), the cations that bind the 18-crown-6 to a significant degree were affected when the amount of 18-crown-6 in the eluent was increased.

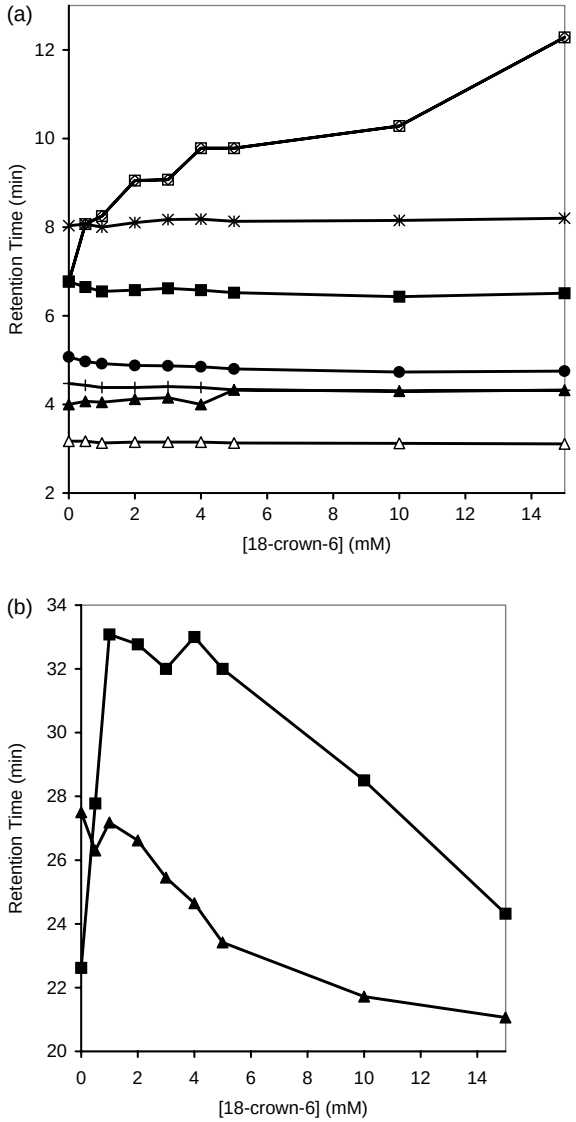

Fig. 3. (a) Separation of cations using different the concentrations of mobile phase 18-crown-6. Column: IonPac CG15 and CS15, Eluent: 10.80 mM H_2SO_4 with varying 18-crown-6 concentration. Flow rate: 1 ml min^{-1}. Detection: suppressed conductivity external water mode when 18-crown-6 was added. Ions: (□) NH_4^+ (2.75 mg l^{-1}); (◇) $CH_3NH_3^+$ (16.50 mg l^{-1}); (✳) Ca^{2+} (5.00 mg l^{-1}); (■) Mg^{2+} (5.10 mg l^{-1}); (●) $(CH_3)_3NH^+$ (17.70 mg l^{-1}); (+) $(CH_3)_2NH_2^+$ (19.30 mg l^{-1}); (▲) Na^+ (2.00 mg l^{-1}); (△) Li^+ (0.54 mg l^{-1}). (b) Effect of the addition of 18-crown-6 to the eluent on K^+ and Sr^{2+} cation retention. Ions: (■) K^+ (5.00 mg l^{-1}); (▲) Sr^{2+} (8.50 mg l^{-1}).

The ions that were affected the most by the addition of 18-crown-6 to the eluent were $K^+ > Sr^{2+} > NH_4^+ = CH_3NH_3^+$ (Fig. 3a and b). The surprising result was that even though the retention time of K^+ *increased* by 10.38 min at 4.0 mM 18-crown-6, the Sr^{2+} retention time *decreased* by 2.9 min. At 15.0 mM 18-crown-6, Sr^{2+} retention decreased by 5.1 min (Fig. 3b). These data show that the effect must result from different mechanisms that influence retention of these two ions.

Several processes influence the retention times of the cations on these columns. First, there is the pairing of the cations with the carboxylate, phosphonate and 18-crown-6 sites on the stationary phase. Second, there is an ion-pairing mechanism between the eluent anion and the analytes. Third, as reported by Ohta [1–5], the 18-crown-6 in the eluent can adsorb onto the stationary phase forming additional retention sites. Fourth, the 18-crown-6 in the eluent can form aqueous complexes with the cations, affecting their association with the stationary phase.

As shown in Fig. 3b, the retention time of Sr^{2+} decreases with increased mobile phase 18-crown-6 concentration. The thermodynamic data (Table 1) show that Sr^{2+} forms a strong complex with 18-crown-6. With its larger effective radius, the resulting complex does not associate with the stationary phase sites as strongly as the unbound cation. Sr^{2+} has a high energy of hydration that inhibits partitioning from the mobile to the stationary phase. Sr^{2+} can associate with the carboxylate, phosphonate and 18-crown-6 stationary phase functional groups or with the mobile phase 18-crown-6. For Sr^{2+}, the competition clearly tips in favor of the mobile phase 18-crown-6, whereas the opposite is true for the K^+ ion.

The balance between solvation and complexation energies for K^+ and NH_4^+, with +1 charge, favors partitioning into the stationary phase. From 1 to 4 mM 18-crown-6, there is a partitioning equilibrium between the stationary and mobile phases causing the retention time for K^+ to remain approximately constant at these concentrations of mobile phase 18-crown-6 (Fig. 3b). However, at 15 mM 18-crown-6, the partition equilibrium for K^+ shifts to the mobile phase causing a reduction in its retention time. As shown in Fig. 3a, at 15 mM 18-crown-6 in the mobile phase, the partition equilibrium for NH_4^+ still tips in favor of the stationary phase. Therefore, the NH_4^+ retention time continues to increase whereas K^+ and Sr^{2+} retention times decrease.

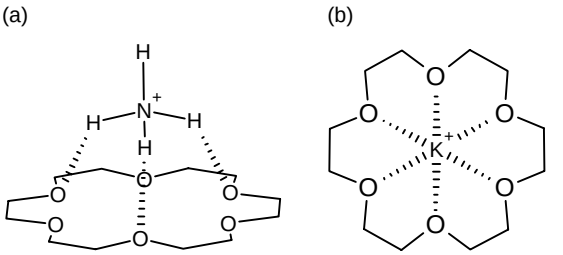

Fig. 4. Binding mechanism of 18-crown-6 to (a) NH_4^+ and (b) K^+.

The difference in the effect on retention times between NH_4^+ and the metal cations may result from the different mechanisms by which 18-crown-6 binds these two types of cations (Fig. 4a and b). Izatt et al. [28] used X-ray crystallographic data to show that NH_4^+ complexes to dicyclohexano-18-crown-6 via hydrogen bonds to the ether oxygen atoms on the macrocycle, sitting atop the ring. However, K^+ and Sr^{2+} bind to the macrocycle by fitting into the inner cavity. As K^+ and Sr^{2+} complex with the mobile phase 18-crown-6, the hydrophilic nature of this complex will prevent it from partitioning into the stationary phase, thereby causing a reduction in retention time. In contrast, as shown in Fig. 4a, the complex of mobile phase 18-crown-6 with NH_4^+ is accessible to adsorb to the stationary phase since the charge center of the complex is well removed from the crown.

It is important to note that the stationary phase 18-crown-6 on the CS15 column causes tailing of the NH_4^+, K^+, and Sr^{2+} peaks. The addition of 18-crown-6 to the eluent of the CS15 column did not cause further tailing of these peaks.

3.3. Optimal mobile phase concentration of 18-crown-6 for the isocratic separation of Na^+ and NH_4^+

The separation of Na^+ and NH_4^+ ions at high concentration ratios was one objective in the development of the IonPac CS15 analytical column [16,17]. Fig. 3a shows how the retention times of Na^+ and NH_4^+ vary with the addition of 18-crown-6 to the eluent. The difference between the retention times of these two ions varied from 2.8 min with no 18-crown-6 in the eluent

to 5.8 min with 4.0 mM 18-crown-6 to 7.9 min with 15.0 mM 18-crown-6. The increased difference in retention time makes it possible to accurately quantify these species at high concentration ratios.

The 10.0 mM 18-crown-6 eluents were used to determine Na^+ and NH_4^+ at concentration ratios of 4000, 8000, and 12,000 to 1 (Fig. 5a–c). As stated earlier, the addition of 18-crown-6 to the eluent moves NH_4^+ to longer retention times while Na^+ is virtually unaffected. Fig. 6 is a chromatogram of the separation of Na^+ and NH_4^+ at a concentration of 60,000 to 1. The longer retention time of NH_4^+ promotes higher

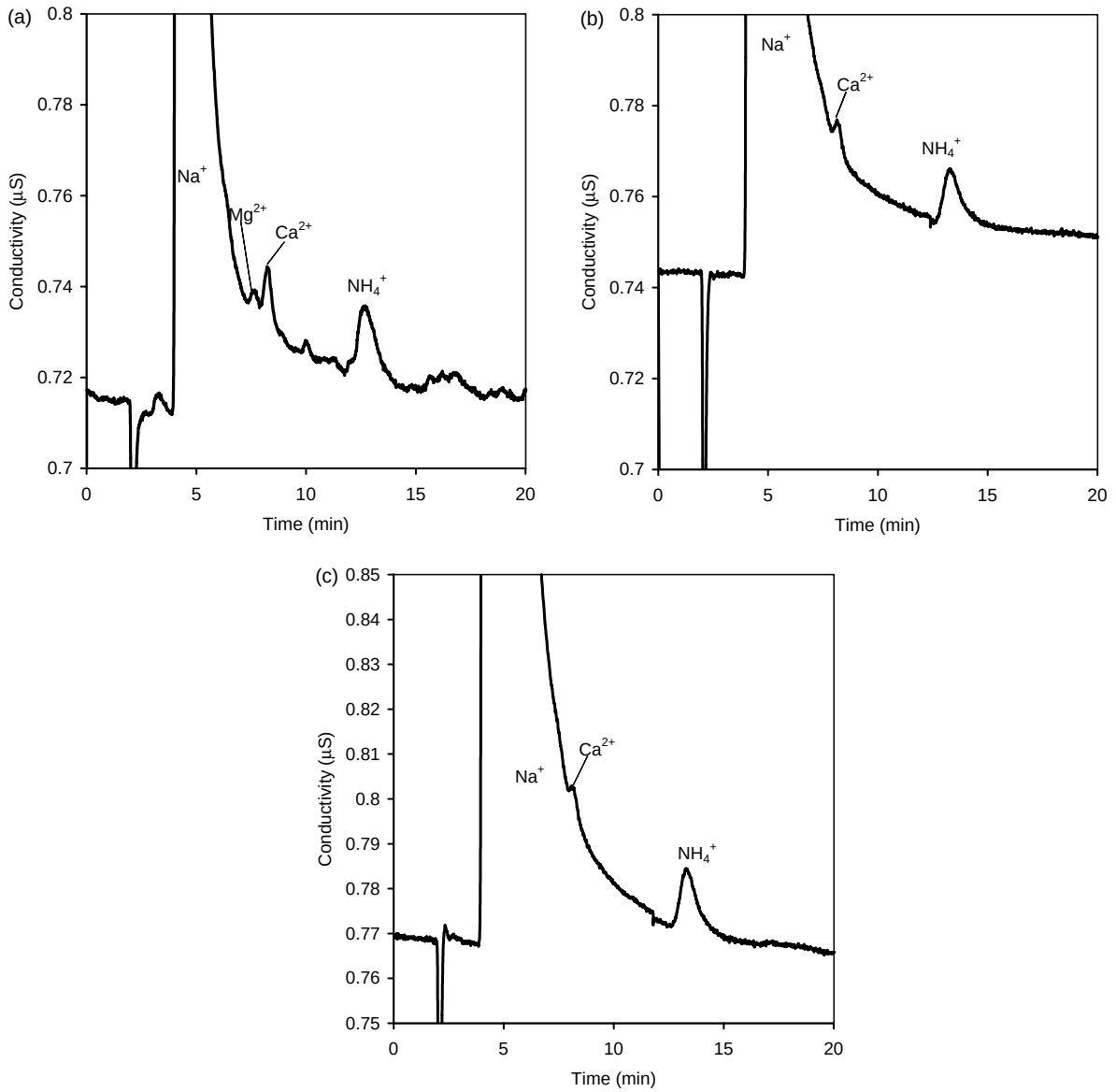

Fig. 5. (a) Separation of Na^+ (100 mg l^{-1}) and NH_4^+ (0.025 mg l^{-1}) concentration ratio 4000:1. Column: IonPac CG15 and CS15. Eluent: 10.80 mM H_2SO_4 and 10.0 mM 18-crown-6. Flow rate: 1 ml min^{-1}. Detection: suppressed conductivity external water mode. (b) Separation of Na^+ (200 mg l^{-1}) and NH_4^+ (0.025 mg l^{-1}) concentration ratio 8000:1. (c) Separation of Na^+ (300 mg l^{-1}) and NH_4^+ (0.025 mg l^{-1}) concentration ratio 12,000:1.

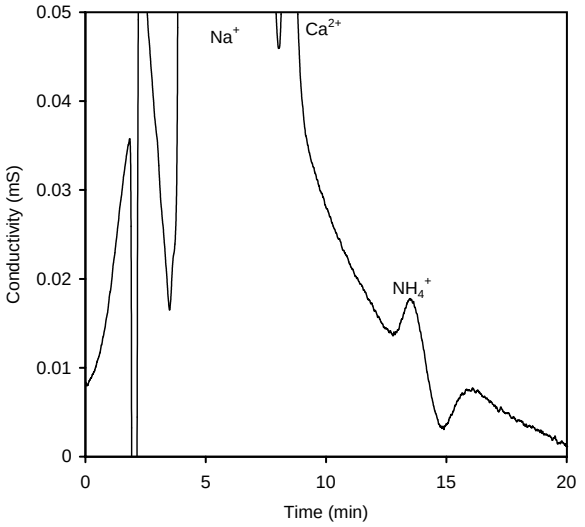

Fig. 6. Separation of Na^+ (600 mg l^{-1}) and NH_4^+ (0.010 mg l^{-1}) concentration ratio 60,000:1. Column: CG15 and CS15. Eluent: 10.80 mM H_2SO_4 and 10.0 mM 18-crown-6. Flow rate: 1 ml min^{-1}. Detection: suppressed conductivity external water mode.

resolution between Na^+ and NH_4^+ yielding separation even at concentration ratios as high as 60,000 to 1.

A calibration curve was used to determine the concentration of NH_4^+ in a sample containing a high concentration of Na^+. The concentration of NH_4^+ in a blind sample was determined to be 0.0933 ± 0.014 mM, at the 95% confidence interval. The actual concentration of the blind sample was 0.0923 mM.

The theoretical detection limits of NH_4^+ are limited by impurities of Ca^{2+} and Mg^{2+}, which elute on the trailing edge of the large Na^+ peak. The detection limit is also limited by an NH_4^+ impurity found in the Na^+ solution detected by injection of a blank sample. Under conditions comparable to those in Fig. 6, the detection limit at 3σ is 0.0023 mM. The range of linearity used for these samples was between 0 and 0.5 mM.

3.4. Column and suppressor durability

After a nine-month period of using mobile phase 18-crown-6, no noticeable harmful effects were observed on either the column or suppressor. In order to further confirm that the crown ether does not damage the suppressor, an eluent of 15.0 mM 18-crown-6 and 10.8 mM H_2SO_4 was pumped continually through the suppressor for approximately 40 days; the total run time was periodically interrupted to change eluent bottles. It is important to note that the concentration of 18-crown-6 used in the eluent was the highest value used, and higher than that which would typically be used. During the 40 days no drifting in the conductivity of the baseline occurred and the background noise level remained minimal. The baseline consistently maintained an absolute conductivity of 1.3 ± 0.2 μS with a background noise level of ±0.004 μS. Periodic injections of the standard cation mixture were also performed to monitor any changes in peak retention and area. As an illustration, the retention time and area of NH_4^+ ion were chosen to report any changes observed in column performance and peak area during the study. The retention time of NH_4^+ ion remained constant at 10.4 ± 0.1 min; however, over a 40-day period, the peak area slowly decreased from a value of 375,000 to 347,000 (arbitrary units), which corresponds to a 7.5% decrease.

After 40 days of continuous running, the ED40 electrochemical detector recorded a suppressor overvoltage, indicating that in order to maintain the selected current, the suppressor control unit needed to supply a voltage higher than permitted. Following the manufacturer guidelines, the suppressor was rinsed for 1 h using a 30% 1.0 M methane sulfonic acid and 70% acetonitrile solution to remove any organic and inorganic contaminants from the suppressor membranes, such as 18-crown-6. Upon completion of rinsing, the suppressor was restored to the normal range and a steady baseline was achieved. A slight decrease in the area under the peaks remained in comparison with the original response, even after rinsing. Since retention times were unaffected, it was concluded that the loss in peak area is related to suppressor, rather than column, operation. Use of a new suppressor confirmed this theory by providing increased peak response similar to that observed before the 40-day study. Small losses in suppressor performance are experienced with continuous use under any circumstances.

Rinsing the suppressor membranes with the acid/acetonitrile solution restored its capabilities by removing many of the membrane adhering contaminants, including 18-crown-6. Our results show that these absorption effects are largely reversible. Use of the crown ether in the mobile phase is compatible with the current cation suppressor and provides good long-term system performance.

3.5. Toxicity and cost

One potential concern with the use of 18-crown-6 is toxicity. The compound is known to be toxic and an irritant, and it affects the nervous system (Table 2). In comparison with some other solvents and organic additives used in chromatography, 18-crown-6 has a relatively low LD50. However, it has a higher LD50 than tetramethylammonium chloride, which is often used in the buffers of capillary electrophoresis. Also, all of the solvents listed in Table 2, with the exception of methanol, target the nervous system. Thus, this compound falls within the range of compounds already in use in such analyses. The cost of 18-crown-6 is not prohibitive, approximately the same per gram as the other organic additives listed in Table 2.

3.6. Application for the determination of NH_4^+ in biological nitrogen fixation process

The advantage of addition of 18-crown-6 to the eluent in this system is illustrated in the measurement of NH_3 production by the enzyme nitrogenase. Traditionally, NH_3 concentrations have been quantified by an indophenol-based method reported by Dilworth et al [29]. The chromatographic method described above significantly reduces analysis time and human error through sample handling because it can be easily automated. Fig. 7 shows the chromatogram from a typical assay of the nitrogenase enzyme activity both with and without the addition of 18-crown-6 to the eluent using the CS15 column. The addition of 15.0 mM 18-crown-6 allowed for the quantification of NH_4^+ in the presence of high concentrations of Na^+ and Mg^{2+}. It was necessary to increase the concentration of 18-crown-6 in the eluent to 15 mM to allow the instrument to return almost to baseline after the large Mg^{2+} peak. As a result of the large Mg^{2+} peak, the NH_4^+ peak was not present in the chromatogram without 18-crown-6 due to its elution near the alkaline earth ions as was shown in Fig. 2. At this concentration, the resolution of the NH_4^+ peak from the Na^+ and Mg^{2+} peaks shifted from a value of zero to 6.19 and 5.65, respectively, as evidenced in Fig. 7. Based on several trials, the concentration of NH_4^+ in a 25 μl nitrogenase study sample determined with this chromatographic method was the same within experimental error as that determined by the traditional indophenol method. However, ion chromatography analysis requires one quarter the time and thirty times less sample.

Table 2
Toxicity of common solvents and organic additives used in ion chromatography and capillary electrophoresis

Eluent additive	Toxicity	Acute health hazard(s)	Target organ
Solvents			
Acetonitrile[a]	ORL-Rat LD50: 2730 mg kg^{-1}	Toxic, lachrymator, irritant	Central nervous system, liver, kidneys, blood, lungs
1,4-Dioxane[a]	ORL-Rat LD50: 4200 mg kg^{-1}	Toxic, irritant	Central nervous system, liver, kidneys
Methanol[a]	ORL-Rat LD50: 5628 mg kg^{-1}	Poison, irritant	Eyes, kidneys
Tetrahydrofuran[b]	ORL-Rat LD50: 1650 mg kg^{-1}	Toxic, irritant	Central nervous system, liver, kidneys
Methylethyl ketone[a]	ORL-Rat LD50: 2737 mg kg^{-1}	Eye irritant, nausea, dizziness	Central nervous system
Organic additives			
18-Crown-6[a]	ORL-Rat LD50: 525 mg kg^{-1}	Toxic, irritant	Nerves
Sodium dodecyl sulfate[b]	ORL-Rat LD50: 1288 mg kg^{-1}	Irritant	Lungs
Tetradecyltrimethylammonium bromide[b]	ORL-Rat LD50: 3900 mg kg^{-1}	Irritant	Lungs, behavioral
Tetramethylammonium chloride[b]	ORL-Rat LD50: 50 mg kg^{-1}	Toxic, irritant	Cardiac, lungs, skin, vascular, behavioral, peripheral nerve and sensation

[a] From [30].
[b] From [31].

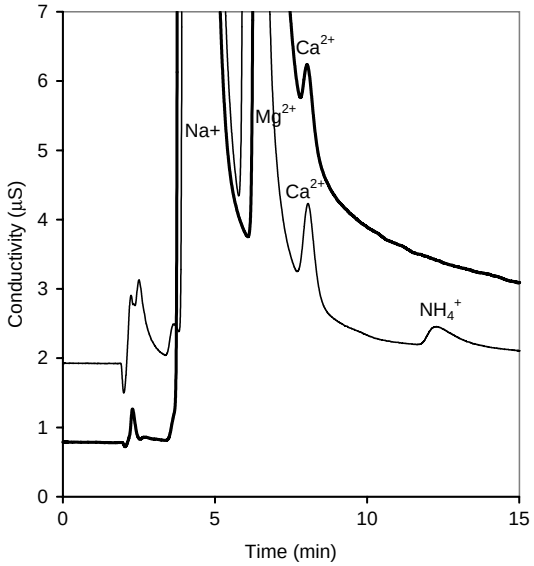

Fig. 7. Quantification of NH_4^+ from the nitrogenase enzyme studies with and without 18-crown-6 in the eluent. Column: CG15 and CS15. Flow rate: 1 ml min^{-1}. Detection: suppressed conductivity external water mode. Concentration of 18-crown-6 in the eluent: (▬) eluent, 10.80 mM H_2SO_4; (—) eluent, 10.80 mM H_2SO_4 and 15 mM 18-crown-6.

4. Conclusion

Mobile phase 18-crown-6 can be used to alter the retention times of some cations and give reproducible analyses even when the column is highly functionalized. The NH_4^+ peak is moved to a longer, yet reasonable, retention time while the Na^+ remains unchanged. This allows for improved resolution between Na^+ and NH_4^+ even at high concentration ratios. Rapid equilibrium and stable operation are achieved when mobile phase 18-crown-6 is used with the CS15 column. Furthermore, mobile phase 18-crown-6 showed minimal effects to the suppressor or column even after extended continuous operation.

The addition of mobile phase 18-crown-6 to the IonPac CS15 column allowed for the determination of NH_4^+ in a nitrogenase sample that contains high concentrations of Na^+ and Mg^{2+}. Analysis of the nitrogenase study sample by IC allowed for the direct, accurate determination of NH_4^+ concentration, decreased the amount of sample required for analysis, and decreased the analysis time.

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